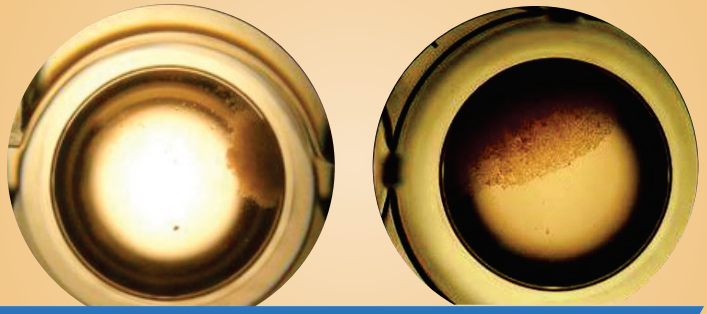


Mouse Lymphoma Assay

Newsletter-7 | July 2021

Introduction

Mouse Lymphoma Assay is an *in vitro* mammalian cell gene mutation test used to detect forward gene mutations (point mutations, frame-shift mutations, small deletions, etc.) and chromosomal events (large deletions, chromosome rearrangements and mitotic recombination).

Mouse Lymphoma TK $^{+/-}$ L5178Y Cell Line is widely used as an alternate assay for checking gene mutation. The assay is applicable for detecting gene mutations for pharmaceuticals, agrochemicals, and chemical substances like nucleuoside analogs and antibiotics which have limitations in Ames test.

Importance of Mouse Lymphoma Assay

L5178Y mouse lymphoma cells are sensitive indicators of mutagenic activity of a broad range of chemical classes. The *TK* mutational system is able to detect base pair alterations, frame shift mutations, small deletions and large-scale chromosomal mutations, recombination, and mitotic nondisjunction.

It is generally recommended as an *in vitro* mammalian gene mutation assay in regulatory test batteries including the U.S. Food and Drug Administration (FDA)/Center for Food Safety and Applied Nutrition (CFSAN), the U.S. Environmental Protection Agency (EPA), Organization for Economic Co-operation and Development (OECD), the International Committee on Harmonization (ICH) and Schedule Y.

Study design

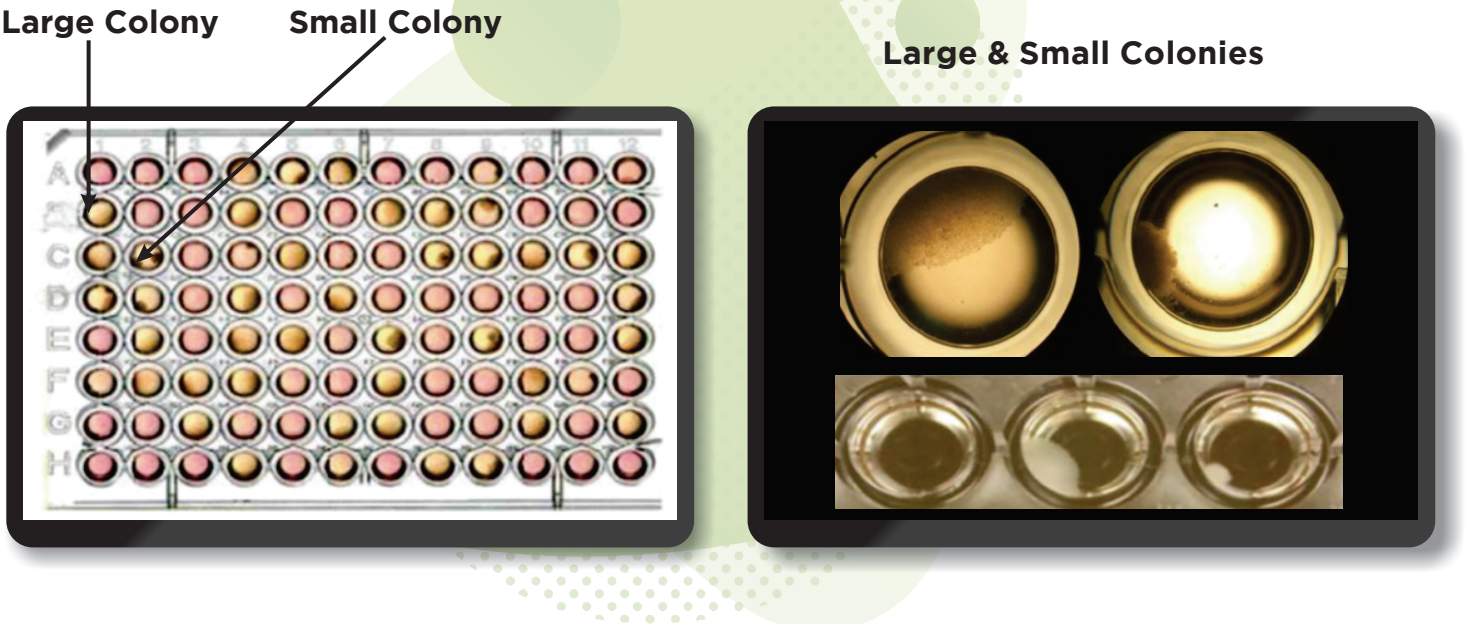
Solubility Test, Precipitation, pH and Osmolality test – Selection of higher concentration and vehicle Cytotoxicity Test - Selection of Test Concentration (Parameters RSG, CE and RTG) Mutagenicity Test- Determination of Mutant Frequency (Parameters RSG, CE, RTG and MF)

Experimental Procedure

Cytotoxicity Test	Mutagenicity Test
Culture Preparation	**Culture Preparation**
Treatment (Day 0)	**Treatment (Day 0)**
Cell Population: 60x10^5 to 1x10^6 cells **Test Item Concentrations:** Chemicals and Agrochemicals: 2 mg/ml, 2 µL/ml or 10 mM whichever is the lower UVCBs: 5 mg/ml	**Cell Population: 60x10^5 to 1x10^6 cells** **Test Item Concentrations:** Chemicals and Agrochemicals: 2 mg/ml, 2 µL/ml or 10 mM whichever is the lower UVCBs: 5 mg/ml

Pharmaceuticals: 1 mM or 0.5 mg/mL Culture Media (RPMI-1640 with 5% serum) **Test Conditions** With and without Metabolic activation (S9) **Exposure:** **Short Term: 3-4 h** **Expression: @ 37±1 °C, 5% CO_2** Change of Media and Adjustment of cells (≈ 3×10^5 cells/mL)	Pharmaceuticals: 1 mM or 0.5 mg/mL Culture Media (RPMI-1640 with 5% serum) **Test Conditions** **Short Term:** With and without Metabolic activation (S9) **Long Term:** Without Metabolic activation (S9) **Exposure:** **Short Term (Phase-I): 3-4 h** (Agrochemicals -pesticides) **Long Term (Phase-I): 24 h** (Industrial Chemicals and Pharmaceuticals) **Expression: @ 37±1 °C, 5% Co_2** Change of Media and Adjustment of cells (≈ 3×10^5 cells/mL)
Day 1 **Counting of viable cells (SG1)** **Adjustment of cells (≈ 3×10^5 cells/mL) for Expression**	**Day 1** **Counting of viable cells (SG1)** **Adjustment of cells (≈ 3×10^5 cells/mL) for Expression**
Day 2 **Counting of viable cells (SG2)** **Plating of cells 1.6 cells/well in 96 well plates** **Incubation: @ 37±1 °C, 5% CO_2 for 10 -12 days**	**Day 2** **Phase-II:** **Counting of viable cells (SG2) Adjustment of cells (≈ 3×10^5 cells/mL) for Expression** **Day 2** **Counting of viable cells (SG2)** **Phase-I: (Plating of cells) 1.6 cells/well in 96 well plates for CE in cloning media 2000 cells/well in 96 well plates for MF in selective media (TFT: 3 µg/mL)** **Incubation: @ 37±1 °C, 5% CO_2 for 10 -12 days** **Day 3** **Counting of viable cells (SG3)** **Phase-I: (Plating of cells)** **1.6 cells/well in 96 well plates for CE in cloning media 2000 cells/well in 96 well plates for MF in selective media** **(TFT: 3 µg/mL)** **Incubation: @ 37±1 °C, 5% CO_2 for 10 -12 days**
Observation: **Empty wells (Ew) for CE**	**Observation:** **Empty wells (Ew) for CE,** **Colony Sizing for Mutation Frequency (MF)** **Large Well (Lw) – Point Mutation** **Small Well (Sw)- Chromosomal Aberration**
End Points: **SG, RSG, CE and RTG**	**End Points:** **SG, RSG, CE and RTG and MF**
Conclusion: Toxicity	**Mutagenic/non-mutagenic**

Observation:



Acceptance and Evaluation Criteria

Acceptance Criteria (As per MLA IWGT)

S.No.	Parameter	Acceptance criteria
1	Mutant frequency	50 – 170 × 10^{-6}
2	Cloning efficiency	65 – 120%
3	Suspension growth	8 – 32-fold (3-4 h treatment) 32 – 180-fold (24 h treatment)

Evaluation Criteria

Global evaluation factor (GEF) of 126 mutants per 10^6 cells.

Conclusion

Mutagenic or non-mutagenic

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About the Author:

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Krishna Kumar Mishra is a Senior Research Officer in the Mutagenicity section. He has a good experience of conducting mutagenicity studies and is actively involved in research validations. He has professional experience of more than 20 years, including academic research, pharmaceutical R&D, KPO and CRO industry.